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Research article

The relationship between semen quality in male infertility clinic patients and bisphenol A : A Chinese cross-sectional study

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ABSTRACT

Bisphenol A (BPA) is a growing concern as an endocrine-disrupting chemical due to its adverse health effects. However, the association between BPA and sperm quality in adult human males remains unclear. The aim of this study was to assess the daily life exposure level of BPA and analyze its correlation with sperm quality in males. Patients who sought treatment in Chinese infertility clinics between May and October 2023 were selected as study subjects. We determined participants' serum BPA content using high-performance liquid chromatography. Sperm count and motility were assessed using a computer-aided sperm analysis system, while sperm morphology was analyzed using an improved Papanicolaou stain. A total of 405 participants, averaging 33.01 ± 5.44 years old, were included. We observed low semen quality among participants in infertility clinics. Principal component analysis was performed for each semen quality index, and three principal components reflecting sperm motility, count, and morphology were extracted. The participants' mean serum BPA level was 6.96 ng/mL. Negative correlations were observed between serum BPA content and total sperm count, sperm density, forward motility rate, and non-forward motility rate. A positive correlation was found between the non-motile sperm rate and the head deformity rate. Morphological abnormalities were the predominant adverse effects observed. Despite low daily life BPA exposure, long-term low-dose exposure in the general population may damage semen quality. This study provides a scientific basis for managing health risks associated with BPA exposure.

1. Introduction

A growing number of studies have confirmed the adverse effects of some substances in the environment on male reproduction, and

Abbreviations: BPA, Bisphenol A; EDC, endocrine-disrupting chemical; HPLC, high-performance liquid chromatograph; WHO, World Health Organization.

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in recent years studies have even clarified their effects on specific parameters of semen, such as quantity, viability and morphology [1, 2]. Many countries have implemented regulations to limit BPA usage; for example, the European Union has banned BPA in baby bottles since 2011, and Canada has declared BPA as a toxic substance [3,4]. However, BPA is still widely used in many parts of the world, leading to significant geographical variations in exposure levels. Geographical variations in BPA exposure have been observed, with levels varying significantly across different regions and populations. For example, studies have reported higher urinary BPA concentrations in North America and Europe compared to Asia and Africa (Lee et al., 2022 [5]; Martinez-Arguelles et al., 2015 [6]). These variations may be attributed to differences in industrial activities, consumer product usage, and regulatory policies regarding BPA in various countries. The implications of these geographical differences for global health are profound, as they may contribute to regional disparities in reproductive health outcomes. For instance, areas with higher BPA exposure may experience a more pronounced decline in semen quality and an increased prevalence of male infertility[7,8]. Understanding and addressing these geographical disparities in BPA exposure is crucial for formulating targeted public health strategies and for ensuring equitable reproductive health outcomes worldwide. Male semen quality has declined globally over recent decades [9], with endocrine-disrupting chemicals (EDCs) identified as pivotal contributors [[10]]. EDCs are exogenous molecules that disrupt the endocrine system homeostasis by inhibiting or activating either estrogen or androgen receptors, directly affecting the human reproductive system and adversely affecting human health [11]. Additionally, EDCs can interfere with hormone synthesis, secretion, metabolism, and binding, thereby affecting physiological functions in animals or humans [12,13]. Bisphenol A (BPA), an EDC [14], commonly used as a raw material in the plastic industry to produce polycarbonate and epoxy resins, is associated with adverse effects on spermatogenesis and male fertility [[10]]. Despite being colorless, transparent, light, durable, and impact-resistant, BPA-containing plastic products, widely used in food packaging, tooth sealants, baby bottles, medical equipment, and daily necessities, raise environmental concerns [15–17]. The United States Environmental Protection Agency reports approximately one million pounds of BPA is released into the environment annually worldwide [14]. BPA can be detected in indoor air, soil, water (drinking, surface, and groundwater), industrial waste, and food products [18]. People are exposed to BPA almost everywhere because of the widespread use of BPA-related products and pollution in their living environment. Tschersich et al. [19] found detectable BPA levels in 95 % and 93 % of urine samples from adults and children, respectively. BPA has also been identified in human serum, plasma, and milk [20], highlighting global concerns about potential health risks associated with long-term BPA exposure. Longitudinal data on BPA levels in human populations have shown a trend of increasing exposure over the past few decades [21]. For instance, a meta-analysis of urinary BPA concentrations from various countries revealed a steady rise in BPA levels from the 1990s to the 2010s [22].

The estrogen activity of BPA was first reported by Dodds and Lawson [23]. Subsequently, the results of cell experiments confirmed that BPA can mimic the binding of estradiol to the estrogen receptor or compete with androgens to bind to the androgen receptor, interfering with the metabolism and synthesis of endogenous hormones and hormone receptors and changing the normal function of the endocrine system [24,25]. Previous animal studies have shown that BPA affects the male reproductive system and can lead to gonadal dysplasia, weight loss of reproductive organs such as testis and epididymis [26,27], decreased sperm quality [28], erectile dysfunction [29], and lag and frequency that influence sexual behavior [30], as well as the development of male reproductive system diseases such as prostate cancer [31,32,33]. These studies demonstrate a negative correlation between increasing BPA concentration in urine and sperm density, number, and motility. Males in contact with BPA were prone to symptoms such as reduced sexual desire, erectile dysfunction, ejaculation difficulties, and diminished overall sexual satisfaction, suggesting that BPA exposure can lead to a decline in semen quality[8,31,34]. Studies have shown that BPA can affect miRNA expression [35–37]. At present, although different scholars have conducted studies on the effects of BPA exposure levels on semen quality in different populations or regions, there has never been a conclusion denying the correlation between the two. These studies were designed to assess the correlations among BPA, steroid hormones, and circulating miRNA concentrations to investigate the potential direct effects of BPA on homeostasis in the testicular environment[25,38].

Several studies have reported the adverse health effects of BPA. For instance, BPA exposure has been associated with various reproductive issues including reduced sperm quality, erectile dysfunction, and increased risk of prostate cancer; [,31–33,39,]. In addition to reproductive health, BPA has been linked to other health issues such as cardiovascular diseases, diabetes, and developmental problems in children [4,40]. A comprehensive review by Vandenberg et al. (2014) [41] highlights the endocrine-disrupting properties of BPA and calls for stricter regulations worldwide. There are numerous regulations in place to restrict the use of BPA, such as the current commentary was triggered by the recent publication by the EFSA Panel on Food Contact Materials, Enzymes and Processing Aids (CEP) of a new draft opinion on BPA: 'Reevaluation of the Risks to Public Health Related to the Presence of Bisphenol A (BPA) in Foodstuffs' (EFSA. 2021). These recent regulatory developments underscore the timeliness and relevance of our study, as they reflect a growing global concern over the health impacts of BPA and a pressing need for research that informs and supports ongoing policy discussions and decisions.

Various exposure routes to BPA exist, including ingestion, skin contact, and respiration. Serum BPA, as an internal exposure marker, effectively reflects individual low-dose multi-route BPA content [42,43]. However, BPA exposure in the general population and its potential impact on reproductive function remain unclear.

In this study, we aimed to assess BPA exposure levels in daily life and analyze the relationship between serum BPA content and sperm quality in an adult male population to provide a scientific basis for controlling the health risks associated with BPA exposure.

In conclusion, the effects of bisphenol A (BPA) on reproductive function have attracted the attention of scholars, and there are studies on the effects of occupational exposure to BPA on male reproductive function, but there are no reports on the effects of BPA on male reproductive function based on daily life exposure levels. Based on the male population of infertility clinic, the present study aimed to investigate the relationship between daily exposure to BPA and male sperm quality and sexual function, to provide direct population-based evidence of the effects of daily exposure to BPA on male reproductive function, and to pave the way for further

investigation of the specific detrimental effects of BPA on male reproductive function.

2. Materials and methods

2.1. Study population

The study subjects comprised adult males seeking treatment at the infertility clinics of Shanxi Bethune Hospital and General Hospital of Tisco from May to October 2023. The participant pool included both healthy and low-fertility individuals (A healthy individual is a man with normal semen quality indicators and normal reproductive function). Inclusion criteria were as follows: couples (male and female partners) who had not taken contraceptives for over 2 years, had no children (both parties included), and resided in the county or city for more than 1 year. Exclusion criteria were as follows: (1) inability to obtain semen specimens; (2) presence of other male reproductive system diseases; (3) family history of disease; (4) chromosomal abnormalities; and (5) engagement in a high-risk occupation potentially affecting semen quality (e.g., direct contact with BPA). The study may have suffered from selection bias because participants were recruited from specific infertility clinics, which may not be representative of the general population. Since clinics may have specific diagnostic criteria or methods for infertility, this can lead to diagnostic biases that affect the representation of participants. The findings may have been influenced by demographic characteristics including age, current residence, degree of education, occupation and alcohol consumption as a behavioural habit that may have been associated with infertility but were not controlled for in the study.

In this study, the rate of semen abnormalities was used as the presenting rate to calculate the sample size. After reviewing the literature, the semen abnormality rate of Chinese men is about 60 %. This survey is a cross-sectional study, according to the sample size calculation formula: $n = t^2PQ/d^2$, so that $d = 0.1, P_\alpha = 0.05$, the sample size required is estimated to be 267 cases. This study resulted in 405 cases of research subjects.

2.2. Data and sample collection

Demographic characteristics, including age, current residence, literacy level, occupation, were obtained through a structured questionnaire from all participants. After completion of the questionnaire, blood and semen specimens were collected from the study subjects. Semen was collected twice every 7–10 d, and the average value of the two measurements was used for analysis. While blood samples were collected from 3 mL of fasting non-anticoagulated venous blood, centrifuged at 3000 rpm for 10 min at room temperature, and the serum was separated from the serum in EP tubes, and then stored at −80 °C in a low temperature refrigerator for examination.

2.3. Determination of serum BPA using high-performance liquid chromatography

To assess BPA exposure, we measured the serum BPA content of the study participants using high-performance liquid chromatography (HPLC). The HPLC method was validated following established protocols, including assessments of accuracy, precision, sensitivity, and specificity.

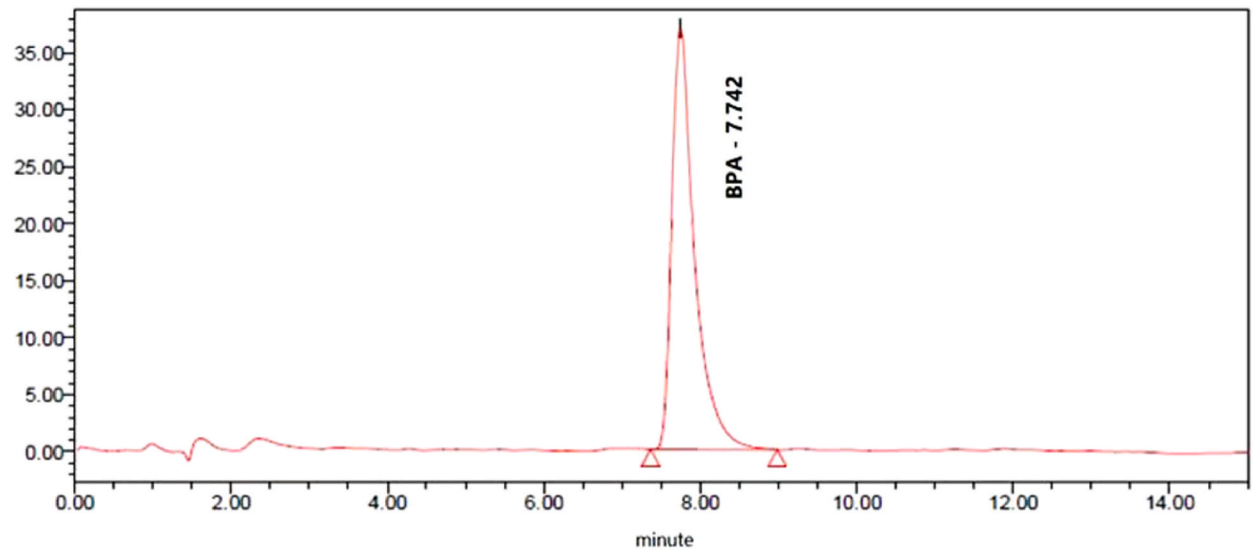

Fig. 1. BPA standard chromatogram for the identification of the HPLC system.

(1) Sample pretreatment: The primary instruments and reagents included a high-performance liquid chromatograph (HPLC) (Waters Alliance E2695, Waters, USA), reverse C18 column (Waters), 2475 fluorescence detector (Waters), BPA standard (Shanghai Biotechnology, China), and β-glucosylanhydrase (Sigma Corporation, Germany). In each colorimetric tube, 0.01 mol/L phosphate buffer (pH = 5) and 20 μL of β-gluconic anhydrase were thoroughly mixed in a 37 °C water bath for 3 h. After cooling to room temperature, an ether:n-ethane (1:1) mixture was added to the tube. Subsequently, 2 mL of the ether:n-ethane (1:1) mixture was added to the original tube, the organic layer was absorbed, and the tubes were placed in a 40 °C water bath and blow-dried with nitrogen prior to testing.

(2) Chromatographic conditions: (i) The chromatography column had an internal diameter of 4.6150 mm and a particle size of 5 μm; (ii) the mobile phase consisted of acetonitrile and ultrapure water in a 40:60 ratio, and (iii) fluorescence excitation and emission wavelengths were 227 and 310 nm, respectively; (iv) chromatography time was 15 min.

(3) Standard curve preparation: Different volumes of the BPA standard application solution were used and diluted with 40 % acetonitrile (10, 20, 25, 50, 100, 250, and 500 μg/L) for determination. The chromatograms of the BPA standards are depicted in Fig. 1. Linear regression of the standard concentration (X) based on the peak area (Y) yielded a standard curve for BPA ($Y = 14981X + 30867$, $R^2 = 0.993$; Fig. 2).

(4) Precision and recovery experiments: Serum samples with known BPA concentrations were analyzed six times Per day according to the detection method described above. The standard curve was used to calculate the concentration, and the relative standard deviation was determined as the precision index (in days). Serum samples with known BPA concentrations were measured at the same time daily for 5 d and used to calculate an inter-day precision indicator. The measurement results showed that the intra- and inter-day precisions were 1.7 % and 6.5 %, respectively. Both values were <10 %, indicating that the precision of this method met the microanalysis requirements. In addition to the above basic and in-house quality requirements, we strictly implemented the fifth edition of the World Health Organization (WHO) Standard Laboratory Manual for the Examination and Processing of Human Semen, and intensive training was provided to laboratory personnel. Meanwhile, a spiker is used in the analysis process and the equipment is calibrated by software analysis to ensure the reliability and reproducibility of sperm quality assessment.

In a parallel experiment, six groups of serum samples with known BPA concentrations were added to a BPA standard solution. Based on the treatment and detection methods outlined earlier, six parallel samples from each serum group were prepared to determine the concentrations of spiked and unspiked serum. Recovery was calculated by dividing the measured serum concentration by the concentration of the BPA standard solution. The recovery in this experiment ranged from 90.2 % to 107.4 %.

(5) Regarding the specific criteria for HPLC, in addition to the basic laboratory requirements, we focused on the validation of the three core indicators of Limit of Detection (LOD) , Limit of Quantitation, (LOQ) , and Relative Standard Deviation (RSD) .

LOD: 0.03 ng/mL, indicating that your method is capable of detecting very low concentrations of BPA, which is important for studying the effects of low-level exposure. LOQ: 0.09 ng/mL, implying that the method is not only able to detect BPA, but also able to perform accurate and reliable quantitative analysis at this concentration level. RSD: 6 %, indicating that the method has good reproducibility, i.e., high consistency of results over multiple measurements. These indicators prove that the detection and analysis method is reliable and necessary to enhance the credibility and transparency of the study.

(6) Injection: Sample residues were fully dissolved in 200 μL of 40 % acetonitrile, with an injection volume of 20 μL. Chromatograms of the blood samples are depicted in Fig. 3.

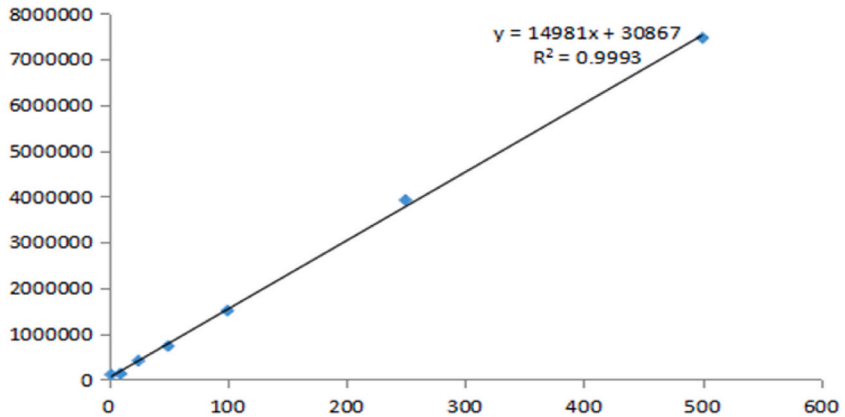

Fig. 2. Standard curve for BPA.

(7) Serum BPA content calculation: The peak area of the standard was added to the equation X = Y − 30867/14981. The BPA content of the sample serum was calculated using the following equation: C = 2.5X = 2.5(Y − 30867/14981).

A calibration curve is constructed from a series of standard solutions with known concentrations, which helps convert the response of the chromatographic peak to the concentration of BPA. Ensure that the selected chromatographic conditions can operate over a wide linear range so that accurate quantitative results can be obtained. The precision of the method is assessed by repeated analysis of the same sample several times, and the precision is usually expressed as the relative standard deviation (RSD), with a low RSD value indicating good reproducibility of the method. Accuracy is assessed by adding a known amount of a standard substance to an unknown sample (labeled recovery experiment), which helps determine whether the method accurately reflects the true concentration of BPA in the sample. Make sure the chromatographic method can distinguish between BPA and other compounds that may be present to avoid cross-contamination or erroneous quantification.

2.4. Sperm quality analysis

Sperm quality analysis was conducted in strict accordance with the World Health Organization (WHO) 5th Edition Laboratory Manual for Human Semen Examination and Treatment Standards. The semen was liquefied and thoroughly mixed for quantity, vitality, and morphological analyses.

(1) Sperm number analysis: (i) Semen volume was calculated at 1 g/mL, and a volume <1.5 mL was considered abnormal. (ii) Total sperm count and density were analyzed using a computer-assisted sperm analysis with a Spanish SCA fully automatic semen quality analysis system. The total sperm count was calculated as the sperm density multiplied by the semen volume. A total sperm count and density of $<39 \times 10^6$/discharge and $<15 \times 10^6$/mL, respectively, were considered abnormal.
(2) Sperm motility analysis: At least five fields were examined for each sample, tracking 200 active sperms, and at least 10 fields were examined in cases of low sperm concentration. VisualThe number of visual fields with a sperm count of 200, non-forward-moving sperms, and motionless sperms were recorded. In the evaluation of sperm motility, sperm with a velocity of 5 μm/s at 37 °C were classified as forward motile sperm, sperm with a velocity <5 μm/s at 37 °C were classified as non-forward motile sperm and nonmotile sperm were classified as immobile. The judgment criteria were as follows: samples with a forward motile sperm rate <32 %, non-forward motile sperm rate <8 %, and immobile sperm rate >60 % were considered abnormal.
(3) Sperm morphology analysis: Sperm morphology was observed using the improved Papanicolaou stain recommended by the WHO. Semen (1 mL) was placed in a centrifuge tube, and 9 mL of phosphate buffer was added. The mixture was centrifuged at 800×*g* for 10 min to remove the supernatant. The supernatant (10 μL) was placed on slides and air-dried for 4 h. The slides were then placed in fixative for 15 s, in dye for 10 s again for 1–5 s, and washed with tap water for 1 min. Subsequently, 2–3 drops were added to seal the specimens, and a microscope (CKX41, Olympus, Japan) was used to calculate the sperm malformation rate. Head deformities included large, small, or double heads with conical, pear, round, or amorphous shapes, or abnormally large vacuoles. Neck deformities included a thick or irregular, angular bending, and abnormally slender neck. Tail deformities included short, multiple, or broken tails, smooth hairpin angular bending tails, and tails with an irregular width and curling. The criteria were as follows: abnormal head, neck, and tail deformities >96 %, >11.5 %, and >8.5 %, respectively. Because a

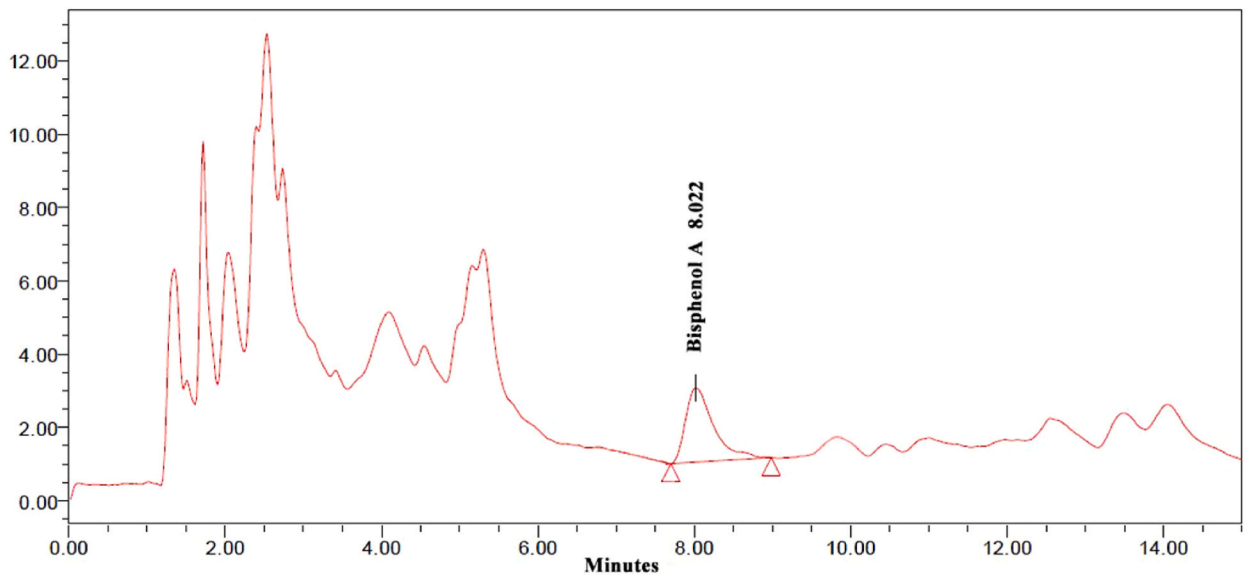

Fig. 3. BPA chromatograms of the blood samples identified by HPLC.

standard for neck and tail deformity rates was not available, the boundary value of the neck and tail deformity rates was determined from a receiver operating characteristic curve using the normal (standard) sperm morphology rate (4 %).

2.5. Data analysis

Quantitative data were used to describe the trend as mean ± standard deviation or median (interquartile range), and analyzed using a *t*-test and analysis of variance (ANOVA); Qualitative data, such as categorical variables, were analyzed using the chi-square test to assess associations between different groups. For correlation analyses, Spearman's rank correlation was used to evaluate the monotonic relationship between BPA exposure levels and semen quality indices, given the potential non-linearity and non-normality of the data. Multiple linear regression models were constructed to explore the relationship between BPA exposure and continuous outcomes of semen quality, Multiple logistic regression was applied to assess the odds ratios of infertility associated with BPA exposure, controlling for the same covariates. Principal component analysis (PCA) was conducted to reduce the dimensionality of the semen quality indices. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity were used to assess the suitability of the data for PCA. Varimax rotation was applied to simplify the interpretation of the components. The first few principal components, which accounted for the majority of the variance, were retained to summarize the original index information. The significance level for all statistical tests was set at $P < 0.05$, and confidence intervals were reported at the 95 % level. Statistical tests were two-tailed, and the software used for all analyses was SPSS version 21.0.

3. Results

3.1. Demographic characteristics of participants

A total of 405 participants were included in this study. The infertility of 163 (40.2 %), 183 (45.1 %), and 59 (14.5 %) participants were caused by male factors, female factors, and both, respectively. This is based on the judgement of the professional judgement, including general physical examination and medical history. The proportion of males with abnormal semen was 54.7 %. The age range of the participants was 23–52 years, with a mean age of 32.89 ± 5.45 years. A chi-square analysis revealed that demographic characteristics including age, current residence, degree of education, occupation and alcohol consumption as a behavioural habit were not associated with infertility. (Table 1).

3.2. Semen quality analysis

In accordance with the WHO 5th edition of the Diagnostic Standards for Male Semen Quality, the semen quality malformation rate among study participants was 55.4 % (Table 2).

3.3. Principal component analysis of the semen quality and the influencing factors

Spearman's rank correlation was used for analysis because the semen quality indices had skewed distributions. The results showed a strong correlation between semen quality indices. The correlation coefficients of the indices are listed in Table 3.

Table 1
Subjects' basic information and infertility-related analysis.

Characteristics	Infertility patients		Normal		X^2	*P*
	N	Percentage (%)	N	Percentage (%)		
Age (years)					1.584	0.453
≤30	86	38.7	79	43.1		
30–	112	50.4	90	49.1		
40–52	24	10.8	14	7.6		
Current residence					0.308	0.601
City proper	185	83.3	147	80.3		
Town and below	37	16.6	36	19.5		
Degree of education					0.878	0.645
Junior high school and below	63	28.3	50	27.3		
High school/technical school	38	17.1	38	20.7		
College degree or above	121	54.5	95	51.9		
Occupation					2.169	0.538
Enterprise/public institution	56	25.2	54	29.5		
Professionals	33	14.8	32	17.4		
Business/Services	44	19.8	35	19.1		
No fixed work	89	40.0	62	33.8		
Alcohol consumption					0.015	0.906
Yes	51	22.9	43	19.3		
No	171	77.0	140	63		

Table 2
Semen quality analysis of the study subjects.

Semen index	Quantitative description					Qualitative description	
	Mean	P 25	P 50	P 75	P95	Abnormal number of cases	Abnormal rate
Sperm quality						222	54.70 %
Sperm number							
Semen volume (mL)	3.53	2.40	3.40	4.50	6.00	13	3.20 %
Sperm density (10^6/mL)	107.40	30.95	65.10	122.30	397.98	49	12.10 %
Total sperm count (10^6/discharge)	420.53	94.45	202.36	413.95	1754.30	46	11.30 %
Sperm motility							
Forward-motile sperm (%)	40.49	27.55	40.70	53.60	70.12	127	31.30 %
Non-forward-motile sperm (%)	24.12	16.85	23.90	30.25	39.50	12	2.90 %
Spermatium (%)	35.36	17.45	33.40	50.05	77.30	60	14.80 %
Sperm form							
Head deformity (%)	95.26	94.00	96.00	99.0	100.0	185	45.6 %
Neck deformity (%)	13.31	8.00	12.0	16.0	26.70	214	52.8 %
Caudal malformation (%)	6.57	3.00	5.00	8.00	16.70	91	22.4 %

In order to reduce multicollinearity and confounding effects, we first performed a principal component analysis to identify and extract the main components affecting the results of the study. Principal component analysis included the following semen quality indices: semen volume (X_1), sperm density (X_2), total sperm count (X_3), forward motility sperm percentage (X_4), non-forward motility sperm percentage (X_5), immobile sperm percentage (X_6), head malformation rate (X_7), neck malformation rate (X_8), and tail malformation rate (X_9). The percentages of the immobile sperm, head deformity rate, neck malformation rate, and tail malformation rate were used for reverse scoring. Characteristic roots, contribution rates, and cumulative contribution rates of each principal component are presented in Table 4.

In this section, we implemented principal component analysis (PCA) to explore the complex relationships between semen indicators (see Table 5). First, we standardised the data to ensure that all variables were on the same scale. Next, we calculated the eigenroots of the covariance matrix and selected the first three principal components based on the Kaiser criterion (eigenroots greater than 1). These three principal components had a cumulative variance contribution of 69.23 %, indicating that they explained most of the variability in the dataset. The first three principal components are as follows:

$$Z1 = 0.0940X1' + 0.4269X2' + 0.3673X3' + 0.4309X4' + 0.3977X5' - 0.5118X6' - 0.1412X7' - 0.2147X8' - 0.0465X9'$$

$$Z2 = 0.4330X1' + 0.4309X2' + 0.5292X3' - 0.3646X4' - 0.1169X5' + 0.3386X6' - 0.0310X7' + 0.1557X8' + 0.2803X9'$$

$$Z3 = 0.1977X1' + 0.0538X2' + 0.1374X3' + 0 + 0.01697X5' - 0.0088X6' + 0.6948X7' + 0.1624X8' - 0.6438X9'$$

By analysing the expressions of the principal components, we found that the first principal component was mainly related to the motility of spermatozoa, especially the percentage of forward-moving spermatozoa (X4), the percentage of non-forward-moving spermatozoa (X5) and the percentage of immobile spermatozoa (X6). The second principal component, on the other hand, was associated with quantitative indicators of spermatozoa, such as semen volume (X1), sperm density (X2) and total sperm count (X3). The third principal component was related to morphological characteristics of sperm, especially head malformation rate (X7) and tail malformation rate (X9).

Table 3
Correlation coefficient between the indicators of sperm quality.

R	Semen volume	Total sperm count	Sperm density	Forward motility sperm	Non forward moving sperm	Immobile sperm	Head deformity	Neck deformity	Tail deformity
Semen volume	1.000	0.390**	0.059	−0.069	−0.086	0.097	0.130*	0.074	0.061
Total sperm count		1.000	0.923**	0.320**	0.533**	−0.475**	−0.204**	−0.127**	−0.013
Sperm density			1.000	0.369**	0.611**	−0.548**	−0.248**	−0.162**	−0.030
Forward motility sperm				1.000	0.313**	−0.908**	−0.412**	−0.332**	−0.351**
Non forward moving sperm					1.000	−0.625**	−0.325**	−0.152**	−0.089
Immobile sperm						1.000	0.446**	0.314**	0.310**
Head deformity							1.000	0.346**	0.229**
Neck deformity								1.000	0.300**
Tail deformity									1.000

Note: *$P < 0.05$, * *$P < 0.001$.

Table 4
Results of the principal component analysis for semen quality.

Principal component	Characteristic root λ_i	Contribution rate (%)	Cumulative contribution rate (%)
Z_1	2.746	30.51 %	30.51 %
Z_2	1.959	21.77 %	52.28 %
Z_3	1.526	16.95 %	69.23 %
Z_4	0.946	10.51 %	79.74 %
Z_5	0.781	8.68 %	88.42 %
Z_6	0.615	6.83 %	95.25 %
Z_7	0.368	4.08 %	99.33 %
Z_8	0.046	0.51 %	99.84 %
Z_9	0.014	0.16 %	100.00 %

Table 5
Component loading matrices for semen quality indicators.

Semen index	Principal component		
	Z_1	Z_2	Z_3
semen volume	0.156	0.600	0.246
total sperm count	0.608	0.734	0.171
sperm density	0.707	0.598	0.067
forward motility sperm percentage	0.714	−0.506	0
non-forward motility sperm percentage	0.659	−0.162	0.021
immobile sperm percentage	−0.234	−0.043	0.865
head malformation rate	−0.848	0.470	−0.011
neck malformation rate	−0.356	0.216	0.202
tail malformation rate	−0.077	0.389	−0.801

3.4. Serum BPA analysis of the study subjects

The serum BPA range was 0.38–21.93 ng/mL, with an average of 6.83 ± 4.29 ng/mL. The F/*t*-test revealed no significant difference in serum BPA concentrations concerning age, current address, education level, or occupation (Table 6).

3.5. Correlation between serum BPA concentrations and various parameters of the semen

Although certain factors (e.g., demographic characteristics including age, current residence, degree of education, occupation) did not show significant associations with the response variables in the preliminary one-way analyses, we still included these factors as covariates in the construction of the linear regression models However, in constructing the linear regression model, we still included these factors as covariates. This was to ensure that all known potential confounders were controlled for in the model, thus providing a more accurate estimate of the effect of the main predictor variables. We used multiple linear regression models to assess the relationship between serum bisphenol A concentrations and sperm quality indicators. We first centred all variables to reduce the effect of multicollinearity. We used t-tests to assess the statistical significance of the regression coefficients and calculated 95 % confidence

Table 6
General profile of the study subjects and serum BPA concentrations.

Variable	BPA (ng/mL)				F/t	*P*
	Mean ± SD	P25	P50	P75		
Age (years old)						
≤30	7.52 ± 4.69	3.93	5.93	10.09	2.626	0.074
30-	6.66 ± 3.97	3.79	5.83	8.44		
40-52	8.06 ± 5.43	4.65	6.23	12.85		
Current residence						
Current urban area	7.12 ± 4.45	3.95	5.71	8.82	0.068	0.794
Town and below	7.27 ± 4.46	3.79	6.61	10.48		
Education level						
Junior high school or below	7.22 ± 4.52	3.75	5.83	9.16	0.196	0.822
High school/Technical school	6.85 ± 4.32	3.88	5.56	6.07		
College degree or above	7.19 ± 4.45	4.06	6.06	8.96		
Occupation						
Professional enterprise/Institution	7.40 ± 4.73	4.26	5.83	8.53	2.221	0.085
Business/Services	6.89 ± 4.74	2.53	4.56	6.30		
Professionals	8.07 ± 4.78	4.36	7.29	11.44		
No fixed work	6.57 ± 3.81	3.93	5.58	8.75		

intervals (CIs) to estimate the uncertainty in the effect sizes. The results showed that Negative correlations were observed between the serum BPA concentrations and total sperm count, sperm density, forward movement rate, and non-forward movement rate. A positive correlation was identified between the immobile sperm and head deformity rates, while no correlation was observed between the serum BPA content and semen volume, neck deformity rate, or tail deformity rate. Specifically, the unstandardised regression coefficients (β) showed that for each unit increase in serum BPA concentration, there was an expected decrease of 23.27 in total sperm count, an expected decrease of 6.68 sperm densities per millilitre, an expected decrease of 1.38 percentage points in forward-motile spermatozoa, and an expected decrease of 0.69 percentage points in non-forward-motile spermatozoa. Standardised regression coefficients (adjusted β) further indicated that BPA had the most significant effect on forward motile spermatozoa, which outweighed other sperm quality indicators (Table 7).

3.6. Correlations between the serum BPA content and each semen quality index

According to the extracted principal components reflecting sperm quality, the first, second, and third principal components reflect sperm motility, number, and morphology, respectively. Simple linear regression analysis revealed a significant negative correlation between serum BPA content and the scores of the first and second principal components. After adjusting for factors affecting sperm motility and sperm count, the negative correlations between the serum BPA content and sperm motility and sperm count could still be observed. The higher the serum BPA content, the lower the sperm motility and count (Table 8).

3.7. Correlations between different serum BPA contents and semen quality

The quartile of the BPA concentration was selected as the threshold, and the serum BPA content was categorized into four grades. We used the first quartile array as a reference to analyze the correlation between BPA concentration and sperm quality in the remaining three groups. The results showed that the risks of a reduced total sperm count and sperm density, forward movement rate, and non-forward movement rate decrease with an increase in the BPA content, and the risks of motionless sperm and head deformity increase. The trend 2 test results suggested dose–effect relationships between BPA content and total sperm count, sperm density, forward movement sperm rate, non-forward movement sperm rate, and head deformity rate, but no correlations were observed with semen volume, neck deformity, or tail deformity (Table 9).

4. Discussion

In this study, we evaluated the semen quality and sexual function of males in infertility clinics and analyzed the relationship between the semen quality and BPA content *in vivo*, from which we provide direct evidence of the effect of BPA exposure on male reproduction. The results provide a theoretical basis for formulating relevant preventive measures and are significant for protecting male health and human society.

Carlsen et al. [44] conducted a meta-analysis revealing a global decline in sperm counts over the past 50 years, marked by an average decrease in sperm volume by 0.7 mL per ejaculation and a reduction in sperm density by 47 $\times$ 10^6/mL. Additional analyses using linear, quadratic, and other models also showed that the semen density of European men has decreased significantly [45,46]. Huang et al. (2017) [47] analyzed the sperm quality in China from 1995 to 2005 and showed that the sperm density and total sperm count significantly decreased, whereas the semen volume did not change significantly. The average semen volume, density, and total number of sperm of the 353 infertility outpatient males in this survey were 3.5 mL, 109 $\times$ 10^6/mL, and 431 $\times$ 10^6 sperm/discharge, respectively. Clinically, sperm motility is considered a more important semen parameter than sperm number. The results of this study

Table 7
Results of the linear regression analysis between serum BPA contents and semen parameters.

Semen index	BPA horizontal				
	β (95 % CI)	adjusted β	SE	t	P
Quality of semen					
Sperm number					
Semen volume	0.025 (−0.01–0.06)	0.079	0.183	1.105	0.112
Total number of sperm	−23.27 (−37.98 to −8.56)	−0.153	0.001	0.282	0.002
Sperm density	−6.68 (−9.34–4.03)	−0.239	0.005	−1.160	<0.001
Sperm motility					
Forward moving sperm	−1.38 (−1.75 to −1.01)	−0.345	0.058	0.001	<0.001
Non-forward-motile sperm	−0.69 (−0.90 to −0.49)	−0.318	0.056	−0.447	<0.001
Spermatium	2.06 (1.61–2.51)	0.410	0.057	1.181	<0.001
Sperm form					
Head deformity	0.26 (0.08–0.45)	0.139	0.030	0.635	0.005
Neck deformity	0.15 (−0.04–0.33)	0.079	0.025	−0.727	0.113
Tail deformity	−0.03 (−0.20–0.15)	−0.014	0.032	−0.874	0.774

Note: *a*.CI, Confidence Interval.*b*.In linear regression analyses, we controlled for multiple covariates including demographic characteristics including age, current residence, degree of education, and occupation, which were included to reduce potential confounding effects.

Table 8
Correlations between each principal component of the sperm quality and serum BPA content.

Principal component	BPA horizontal				
	β (95 % CI)	adjusted β	SE	t	P
Sperm motility	−13.40 (-19.93 to −6.86)	−0.197	3.324	−4.030	<0.001
Sperm number	−13.77 (-22.53 to −5.02)	−0.152	4.454	−3.092	0.002
Sperm form	−2.22 (-4.28–0.02)	−0.096	1.093	−1.944	0.053

Note: CI, Confidence Interval.

Table 9
Correlations between different serum BPA contents and the sperm quality.

Semen index	*BPA level (ng/mL) or (95 % CI)*				χ^2_{rend}	*P*
	0.38–3.79	3.79–5.79	5.79–8.68	8.68–21.93		
Sperm quality	1	3.29 (1.73–6.24)	6.74 (3.46–13.10)	11.41 (5.63–23.13)	58.32	<0.001
Sperm number						
Liquid essence	1	0.13 (-0.11–0.36)	0.06 (-0.15–0.26)	0.05 (-0.19–0.28)	4.534	0.209
Total number of sperm	1	0.01 (-0.14–0.14)	−0.10 (-0.29–0.10)	0.22 (-0.10–0.54)	39.59	<0.001
Sperm density	1	−0.03 (-0.24–0.11)	0.04 (-0.18–0.25)	−0.06 (-0.39–0.27)	50.53	<0.001
Sperm motility						
Forward moving sperm	1	−0.07 (-0.28–0.14)	0.14 (-0.08–0.36)	0.06 (-0.11–0.24)	51.42	<0.001
Non-forward-motile sperm	1	−0.05 (-0.24–0.15)	0.07 (-0.15–0.28)	0.04 (-0.15–0.28)	13.19	0.004
Spermatium	1	−0.02 (-0.23–0.19)	0.04 (-0.16–0.24)	0.09 (-0.07–0.24)	39.47	<0.001
Sperm form						
Head deformity	1	−0.16 (-0.67–0.35)	−0.02 (-0.21–0.17)	−0.05 (-0.63–0.53)	32.85	<0.001
Neck deformity	1	0.05 (-0.21–0.32)	−0.08 (-0.26–0.10)	−0.21 (-0.42–0.01)	7.57	0.056
Tail deformity	1	−0.05 (-0.31–0.21)	−0.01 (-0.12–0.11)	−0.05 (-0.30–0.19)	3.85	0.278

Note: CI, Confidence Interval.

show that the average forward sperm rate is 31.3 % and that the primary sperm abnormalities were morphological, which is consistent with previous findings that abnormal sperm morphology contributes to 30–40 % of infertility cases [48,49]. Given the negative correlations observed between serum BPA content and sperm count, motility, and morphology, these findings have significant implications for countries with higher BPA exposure levels. In such countries, the prevalence of male infertility due to impaired semen quality may be higher, posing a substantial public health challenge.

To assess BPA exposure, we measured the serum BPA content of the study participants, reflecting the level of BPA exposure through multiple pathways. The average serum BPA concentration was 6.83 ng/mL, ranging from 0.38 to 21.93 ng/mL. In a study of 153 Italian adult males, BPA was detected in 60 % of the participants, with a mean detection concentration of 5.7 ng/mL [50]. In the United States, the median serum BPA concentration in male patients was 0.48 ng/mL, and BPA concentrations range from 0 to 22.7 ng/mL. Beutel et al. [51]· Tested serum BPA concentrations in 245 cases from the general population, among which 120 were detectable and ranged from 0 to 15.9 ng/mL. In the present study, the serum BPA content in the general population was higher than the average level reported in recent studies, which may be related to regional differences, living habits, and population structure. The negative correlations observed between serum BPA content and these parameters suggest that in countries with higher BPA exposure levels, there may be a greater risk of reduced semen quality, which could lead to an increased incidence of male infertility. In addition, HPLC analysis results are closely related to the establishment of the system, which may also be related to the differences in parameter settings between detection systems.

The results of both *in vitro* and *in vivo* experiments reveal the adverse effects of BPA on sperm, affecting its quality [52,53]. Semen volume, total sperm count, and sperm density serve as key indicators for assessing sperm count. The total semen volume reflects the secretory capacity of the gland, the total number of sperm reflects the spermatogenesis of the testis, and the degree of patency after the testes and sperm density is a crucial predictor of male fertility [54]. In this study, negative correlations were observed between serum BPA content and sperm count. As serum BPA concentration increased, both sperm density and total sperm count decreased. This suggests that daily BPA exposure in the general population might be linked to a reduction in sperm count. The negative correlation between serum BPA content and sperm motility observed in our study further supports the notion that BPA exposure may impair sperm motility, particularly in countries with higher BPA exposure levels, potentially exacerbating the public health burden of male infertility.

Sperm motility is often used as an early, sensitive indicator for evaluating male sexual reproductive toxicity. Knez et al. [55] measured urine BPA concentrations in 149 men with infertility and reported that their urine BPA concentrations were negatively correlated with sperm motility. Mendiola et al. [45] analyzed the relationship between the urine BPA concentration and sperm motility in males with normal fertility and observed a negative correlation between them. The results of this study showed that serum BPA content was negatively correlated with the principal component reflecting sperm motility. Negative correlations were observed between serum BPA concentration and forward motile and non-forward motile sperm rates, and a positive correlation was observed between the BPA content and non-motile sperm rate. BPA exposure may impair sperm motility, resulting in a decrease in the motile

sperm count and an increase in the number of immobile spermatozoa, thereby affecting male fertility.

Sperm morphology plays a crucial role in the conception process, and any form of malformation may result in adverse reproductive outcomes such as infertility, malformation, or miscarriage. Animal experiments indicated an increase in malformed spermatozoa in mice after 2 weeks of exposure to 10–40 mg/kg BPA [56]. Xiaoyu and Hong [33] reported a significant difference in normal sperm morphology rates between occupational and control populations. The results of this study revealed a positive correlation between the serum BPA content and sperm head malformation rate. These findings suggest that daily exposure of the general population to BPA may be related to a decline in normal sperm morphology. Currently, related studies on sperm neck and tail malformations have not been published; however. Correlations between serum BPA concentrations and sperm neck and tail malformation rates were studied; however, no significant correlation was observed, likely because the sperm head is the core component of sperm (including the nucleus and acrosome), which is more susceptible to external stimuli. BPA interacts with sperm DNA and interferes with the expression of related genes, resulting in induced sperm aberration, mainly in the sperm head.

Studies in the last five years have demonstrated the potential impact of BPA on the reproductive health of specific populations, such as Chinese men [57,58]. These studies are innovative in that they not only focus on a single exposure to BPA, but also incorporate the assessment of multi-chemical exposures and utilize local data, thus providing a more comprehensive view of the effects of BPA on a global scale [41,59,60]. Meanwhile, a related study revealed that BPA may affect male reproductive health through endocrine disrupting mechanisms by evaluating the relationship between urinary BPA concentrations and semen quality parameters, which is consistent with ours in that both used biomarkers to assess the reproductive toxicity of BPA [61], but our study went a step further in that we were able to analyze the relationship between BPA and semen quality more directly through an experimental study in which blood and semen were collected from a population of fertility clinics. Causal relationship between BPA and semen quality [2]. At a time when a great deal of the current relevant literature in this field mostly uses only epidemiological surveys and cross-sectional studies [39,62,63], we attempted to combine this with experimental studies, which will provide a deeper mechanistic understanding in directly analyzing the causal relationship between BPA and semen quality through experimental and statistical methods. Subsequent exploration will continue in terms of in-depth combination [64,65]. Meanwhile, we plan to accommodate multiple samples including biological samples (e.g., urine and blood samples) and environmental samples (e.g., long-term monitoring of BPA levels in municipal wastewater) for synergistic studies in future studies [66]. This approach will allow for a more scientific and comprehensive assessment of the risk of exposure to the entire population.

However, this study has some limitations that should be acknowledged. First, the study population was recruited from an infertility clinic, which, while ensuring the necessary conditions for semen sample collection and real-time analysis of sperm quality parameters, may lead to an overrepresentation of low-fertility males [2]. This selection bias means that the findings may not be fully generalizable to the broader population [61]. To address this limitation, future studies should include a larger and more diverse sample from the general population to validate our results [41]. Second, the assessment of BPA exposure was based on a single blood sample from each participant. This method may not accurately reflect chronic BPA exposure levels due to variations in BPA metabolism and clearance rates [5,60]. Future research should employ longitudinal designs with repeated measurements of BPA and include various biomarkers, such as urinary BPA concentrations and BPA-glucuronide levels, to provide a more comprehensive assessment of BPA exposure [64]. Third, while our study focused on serum BPA levels, it is important to consider that BPA exposure can occur through multiple pathways, including ingestion, inhalation, and dermal contact [67]. Future investigations should explore these different exposure routes and their combined effects on semen quality and sexual function [57,59]. Lastly, although we observed significant associations between BPA exposure and various semen parameters, the underlying biological mechanisms remain unclear [68]. Future studies should aim to elucidate these mechanisms through advanced molecular and genomic analyses, which could provide deeper insights into how BPA impacts male reproductive health at the cellular and molecular levels [65]. By addressing these limitations and exploring these avenues for further research, we can obtain a more balanced and comprehensive understanding of the impact of BPA on male reproductive health [2,58,69]. In addition, although not statistically significant, there may be a potential association between BPA and sperm abnormality rate, which needs to be further explored in future studies [41,60].

5. Conclusions

Our study reveals a concerning decline in the semen quality of males in infertility clinics, primarily characterized by morphological malformations. Notably, the serum BPA content exhibits negative correlations with crucial sperm parameters, including total sperm count, sperm density, and sperm motility (both forward and non-forward motility rates). Furthermore, positive correlations are observed between the non-motile sperm rate and head malformation. These results suggest that long-term low-dose exposure to BPA may significantly compromise semen quality in the general population.

Ethical approval

This study was approved by the Ethical Review Committee of Taiyuan Iron and Steel Group General Hospital (K202312).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

Patients signed informed consent regarding publishing their data.

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CRediT authorship contribution statement

Zhiqiang Tian: Writing – original draft, Methodology, Conceptualization. **Zhiwen He:** Project administration, Investigation, Data curation. **QingQuan Zhang:** Writing – original draft, Resources, Funding acquisition. **Ling Ding:** Methodology, Formal analysis, Data curation. **Li Song:** Writing – original draft, Methodology, Data curation. **Ruimin Ren:** Writing – review & editing, Investigation, Data curation. **Kai Tan:** Visualization, Project administration, Formal analysis. **Shifu Cao:** Project administration, Data curation. **JinTao Wang:** Writing – review & editing, Methodology, Conceptualization. **Baolong Pan:** Resources, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e35982.

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